## WEST

## End of Result Set

Glu-Lys-Lys, Glu-Ile-Thr,

## Generate Collection

File: USFT 13: Entry 1 of 1 Jan 16, 1999 US-PAT-NO: 5863894 DOCUMENT-IDENTIFIER: US 8863894 A TITLE: Nematode-extracted anticoagulant protein DATE-ISSUED: January 38, 1999 INVENTOR-INFORMATION: CITY STATE ZIP CODE COUNTRY NAME Vlasuk; George Phillip Carlsbad CAN'AN/AStanssens; Fatrick Eric Hugo St-Martens-Latem :: A N A BEX Messens; Joris Hilda Lieven :1. A Antwerp N A ВЕХ Lauwereys; Marc Jozef :: A Haaltert N A BEX Laroche; Yves Rene Brussels 11. A N A BEX Jespers; Laurent Stephane Tervuren :1. A N A BEX Gansemans; Yannick Georges Jozef Bredene 11, A N. A BEX Moyle; Matthew Escondido  $CI_{\Lambda}$ N.A N/A Bergum; Peter W. San Diego  $\mathbb{C} I_{\Lambda}$ N. A N/AUS-CL-CURRENT: <u>514/12</u>; <u>530/324</u>, <u>530/350</u> CLAIMS: We claim: 1. An isclated protein having Factor Ma inhibitory activity and having one or more MAP domains, wherein each NAP domain includes the sequence: Cys-Al-Cys-A2-Cys-A3-Cys-A4-Cys-A5-Cys-A6-Cys-A7-Cys-A3-Cys-A 9-Cys-A10, wherein (a) Al is an amino acid sequence of 7 to 8 amino acid residues; (b) A2 is an amino acid sequence; (c) A3 is an amino acid sequence of 3 amino acid residues; (d) A4 is an amino acid sequence; (e) AS is an amino abid sequence of 3 to 4 amino abid residues; -f) A6 is an amino acid sequence; 'g) A7 is an amin: abid residue; h) A8 is an amine acid sequence of 11 to 12 amine acid residues; i) A9 is an amino acid sequence of 5 to 7 amino acid residues; and (j) AlO is an amino acid sequence; wherein each of A2, A4, A6 and A10 has an independently selected number of independently selected amino acid residues and each sequence is selected such that each NAP domain has in total less than about 120 amino acid residues and wherein said isolated protein is derived from a hematophagous nematode species. 1. The pritein of claim 1, wherein A3 has the sequence Glu-A3.sub.a -A3.sub.b, wherein A3. sub.a and A3. sub.b are independently selected amino acid residues. 3. The protein of claim 1, wherein Ab has the sequence Glu-Ab.sup.a -Ab.sup.p, wherein A3.sub.a is selected from the group consisting of Ala, Arg, Pro, Lys, Ile, His, Leu, and Thr, and As.sub.b is selected from take group consisting of Lys, Thr, and Arg. 4. The protein of claim 3, wherein A3 is selected from he group consisting of Glu-Ala-Lys, Glu-Arg-Lys, Glu-Pro-Lys,

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Glu-His-Arg,
 Glu-Leu-Lys, and
 Glu-Thr-Lys.
 5. The protein of claim 1, wherein A4 is an amino acid equence having a net anionis
 charge.
 6. The protein of claim 1, wherein AT is Mal.
T. The protein of claim 1, wherein AT is The.
8. The protein of claim 1, wherein At includes the amin. Atin sequence -Af.sub.a
 -A8.sub.b -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.y -(SEQ. 10. NO. 88),
 wherein
 (a) At.sub.a is the first amino acid residue in At,
 (b) at least one of Afisubia and Afisubib is selected from the group consisting of
 Glu or Asp, and
  [5] Al.sub.c through Al.sub.g are independently selected amino acid residues.
 9. The protein of claim \hat{\epsilon}_{\star} wherein
 (a) Af.sup.a is Glu or Asp,
 (b) Af.sub.b is an independently selected amino acid residue,
 (c Af.sur.c is Gly,
 (d) Aft.sum.d is selected from the group consisting of Phe, Tyr, and Leu,
 (e Af.suc.e is Tyr,
 (f AB.sub.f is Arg, ani
 (g Ad.sur.g is selected from Asp and Asn.
10. The protein of claim 9, wherein -A8.sub.c-A 8.sub.d-A 8.sub.e -A8.sub.f -A8.sub.q
 - is selected from the group consisting of
 Gly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 69],
 Sly-Phe-Tyr-Arg-Asn (SE). ID. No. 70),
 31y-Tyr-Tyr-Arg-Asp [SE]. IE. NO. 71
 3ly-Tyr-Tyr-Arg-Asn \{SE\}. II. No. 72], and
 Gly-Leu-Tyr-Arg-Asp (SE). IF. NO.
 11. The protein of claim 8, wherein (a A8.suc.a is an independently selected amino acid residue,
 (r A8.sur.b is 31u or Asp,
 (a A8.sub.c is Bly,
(d) A8.sub.d is selected from the group consisting of Phe, Tyr, and Leu,
(e) A8.sub.e is Tyr,
(f) A8.sub.f is Arg, and
(g' A8.suk.g is selected from Asp and Asn.
12. The protein of claim 11, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A 8.sub.f
-All.sub.g - is selected from the group consisting of
Gly-Phe-Tyr-Arg-Asp [SET. ID. No. 69],
Gly-Phe-Tyr-Arg-Asm [SEQ. ID. NO. 70],
Gly-Tyr-Tyr-Arg-Asp [SE]. ID. NO. 71],
Gly-Tyr-Tyr-Arg-Asn [SEQ. II. No. 72], and
Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 73].
13. The protein of clair 8, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g
- is selected from the group consisting of
Gly-Phe-Tyr-Arg-Asp [SEq. II. No. 69],
3ly-Phe-Tyr-Arg-Asn [SE.. II. ND. 70],
Sly-Tyr-Tyr-Arg-Asp [SE. II. No. 71],
Sly-Tyr-Tyr-Arg-Asp [SE. II. No. 72], and
Sly-Leu-Tyr-Arg-Asp [SE. II. No. 73].
14. The protein of clair 1, wherein AlO includes an amino acid sequence selected from
the group consisting of
Glu-Île-Île-His-Val [SE]. II. ND. 74],
Asp-Île-Île-Met-Val [SE]. II. NI. 75],
Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. 76], and Met-Glu-Ile-Inr [SEQ. ID. NO. 77].

15. The protein of claim 14, wherein A10 includes the amino acid sequence
Glu-Ile-Ile-His-Val [SEq. ID. 74].
16. The protein of claim 15 naving a NAP domain with an amino acid sequence
substantially the same as that of AcaNAP5 (SEQ. ID. NO. 40) or AcaNAP6 (SEQ. ID. NO.
41].
17. The protein of claim 14, wherein AlS includes the amino acid sequence \frac{1}{2}
Asp-Ile-Ile-Met-Mal (SE), ID. No. 78).
18. The protein of claim 14, wherein A10 includes the amino acid sequence
Phe-Ile-Thr-Fhe-Ala-Pro [SEq. 1D. NO. 76].
19. The protein of claim 14, wherein Alf includes the amino acid sequence
Met-Gla-Tie-Tie-Thr (SEQ. ID. NO. 70)
20. The protein of claim 1, wherein said nemat de species is selected from the or up
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consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma ducdenale,
Negator americanus, and Heligomosomoldes polygyrus.
21. The protein of claim 1, wherein
 a' A3 has the sequence Glu-A3.sub.a -A3.sub.b, wherein A3.sub.a and A4.sub.b ar4
independently selected amino acid residues;
 (p) A4 is an amine acid sequence having a net anience charge;
 (a) AT is selected from the group consisting of Val and Ile;
 (d) Ax includes an amino acid sequence selected from the group consisting of
Gly-Pne-Tyr-Arg-Asp [SE2. ID. NO. 69],
Gly-Pne-Tyr-Arg-Asn [SE2. ID. NO. 70],
Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 71],
Gly-Tyr-Tyr-Arg-Asp [SEQ. 10. NO. 1], Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 72], and Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 73]; and
(e) All includes an amino sequence selected from the droup insisting (
Glu-Ile-Ile-His-Val (SE). ID. N. . 74
Asp-Ile-Ile-Met-Val (SEQ. ID. N. . 75
Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. 76], and Met-Glu-Ile-Thr [SEQ. ID. NO. 77].
22. The protein of claim 21 having a NAP domain substantially the same as NAP domains
selected from AcaNAP5 (SEQ. ID. NO. 40) and AcaNAP6 (SEQ. ID. NO. 41).
23. The protein of claim 22, wherein said nematode species is selected from the group
tonsisting of Ancylostoma caninum, Ancylostoma devlanicum, Ancylostoma duodenale,
Medator americanus, and Heligomosomoides polygyrus.
24. The protein of claim 1, wherein
(a) Af is selected from the group consisting of
Glu-Ala-Lys,
Glu-Arg-Lys,
Glu-Pro-Lys,
Glu-Lys-Lys,
Glu-Ile-Thr,
Glu-His-Arg,
Glu-Leu-Lys, and
Glu-Thr-Lys;
(b) A4 is an amino acid sequence having a net anionic charge;
(c) A7 is Val or Ile;
(d) A% includes an amino acid sequence selected from the group consisting of
Af.sub.a -A3.sub.c -Gly-Phe-Tyr-Arg-Asp [SEQ. ID. MO. 78],
Aalsukla -Aalsubic -Gly-Ene-Tyn-Arg-Ash (SE). ID. 80.
At.suk.a -At.sub.c -Sly-Tyr-Tyr-Arg-Asp (SEQ. ID. NO. 80),
At.sub.a -At.sub.c -Gly-Tyr-Tyr-Arg-Asp (SEQ. ID. NO. 81), and
A8.sub.a -A8.sub.t -Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 82], wherein at least one of
At.sub.a and A3.sub.b is Glu or Asp;
(e) A9 is an amind adid sequence of five amino adid residues; and
(f) A10 includes an amino acid sequence selected from the group consisting of
Glu-Ile-Ile-His-Val [SEQ. ID. MO. 74], Asp-Ile-Ile-Met-Val [SEQ. ID. MO. 78],
Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. ^{7}6], and Met-Glu-Ile-Ile-Thr [SEQ. II. NO. ^{7}6].
If. The pritein of claim 14 having a NAP domain substantially the same as NAP domains
selected from AcaNAPS [SE2. ID. NO. 40] and AcaNAPS [SEQ. ID. NO. 41].
10. The pritein of claim 14, wherein said nematode species is selected from the group
consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma duodenale,
Medator americanus, and Heligomosomoides polygyrus.
27. A pharmaceutical composition comprising the protein of claim 1.
26. A pharmaceutical composition comprising the protein of claim 21.
14. A pharmaceutical composition comprising the protein of claim 24.
3) A method of inhibiting blood coagulation comprising administering a protein of
claim I with a pharmacout cally acceptable carrier.
31. A method of inhibitiny blood poadulation comprising administering a protein of
claim 11 with a pharmageurically acceptable carrier.
30. A method of inhibiting blood coagulation comprising administering a protein of
claim 24 with a pharmaceutically acceptable carrier.
FB. A protein of claim 1, wherein said protein has two NAP domains.
34. A protein of claim 21, wherein said protein has two NAP domains.
35. A protein of claim 24, wherein said protein has two NAP domains.
36. A method of treating a pathologic condition characterized by abnormal thrombysis.
by preventing or decreasing said abnormal thrombosis, which comprises administering a
protein of claim 1.
37. A method according to claim 36 wherein said plathologic condition is disseminated
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intravascular ccagulopathy.

- 39. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or Decreasing said abnormal thrombosis, which comprises administering a protein of claim 21.
- 39. A method according to claim 38 wherein said pathologic condition is disseminated intravascular coagulopathy.
- 41. A method of treating a pathologic condition characterized by abnormal thromassis by preventing or decreasing said apnormal thromassis, who we explose a monostreing a protein of claim 24.
- 41. A method according to claim 40 wherein said pathologic condition is disseminated intravascular coagulopathy.
- 42. An isolated protein having Factor Ma inhibitory activity selected from the group consisting of AcaNAP5 [SEQ. ID. NO. 40] and AcaNAP6 [SEQ. ID. NO. 41].
- 43. A pharmaceutical composition comprising a protein selected from the group consisting of AbaNAPS [SEQ. ID. NO. 40] and AbaNAPS [SEQ. ID. NO. 41].
- 44. A method of inhibiting blood coagulation comprising administering a protein selected from the group consisting of AcaNAP5 [SEQ. ID. NO. 40] and AcaNAP6 [SEQ. ID. NO. 41].
- 45. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 42.
- 46. A method according to claim 45 wherein said pathologic condition is disseminated intravascular coagulopathy.